FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **ABLE-0021**

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR): **10/088780**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/03605	**20 September 2000**	**20 September 1999**

TITLE OF INVENTION

MONOCLONAL ANTIBODY 3F1H10 NEUTRALISING VHSV (VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS)

APPLICANT(S) FOR DO/EO/US

SECOMBES, Christopher John, CUNNINGHAM, Charles and LORENZEN, Niels

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau)
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1 821 - 1 825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S C. 154(d)(4)
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

1) Courtesy copy of the International Application;
2) Return post card.

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/088780	PCT/GB00/03605	ABLE-0021

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	52 - 20 =	32	x $18.00	**$576.00**	
Independent claims	4 - 3 =	1	x $84.00	**$84.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,550.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,550.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,550.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,550.00**	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-1619** A duplicate copy of this sheet is enclosed.

d. ☒ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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38,350
REGISTRATION NUMBER

March 20, 2002
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**ABLE-0021**
Inventors:	**Secombes et al.**
Serial No.:	**Not yet assigned**
Filing Date:	**Herewith**
Examiner:	**Not yet assigned**
Group Art Unit:	**Not yet assigned**
Title:	**Monoclonal Antibody 3F1H10 Neutralising VHSV (Viral Haemorrhagic Septicaemia Virus)**

"Express Mail" Label No. **EV051547277US**
Date of Deposit **March 20, 2002**

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1 10 on the date indicated above and is addressed to the U.S. Patent and Trademark Office, PO Box 2327, Arlington, VA 22202

By Kathleen A. Tyrrell
Typed Name: **Kathleen A. Tyrrell, Reg. No. 38,350**

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

PRELIMINARY AMENDMENT

Please enter the following amendments into the record.

In the Claims:

Please cancel claims 19 and 20.

Please amend the claims as follows:

1. (Amended) A composition for protection of an animal against a disease-causing agent, the composition comprising a non-

infectious nucleic acid construct encoding a recombinant antibody to that agent.

2. (Amended) A composition according to claim 1 wherein the animal is selected from a mammal or a fish.

3. (Amended) A composition according to claim 1 wherein the animal has a deficient immune system.

4. (Amended) A composition according to claim 1 wherein the disease-causing agent is selected from a pathogen, an allergen or a toxic substance.

5. (Amended) A composition according to claim 1 wherein the protection is prophylactic.

6. (Amended) A composition according to claim 1 wherein the encoded recombinant antibody is derived from an antibody raised against the disease-causing agent.

7. (Amended)A composition according to claim 1 wherein the encoded antibody molecule comprises variable domains of immunoglobulin Heavy and Light chain genes linked together by a linker sequence.

8. (Amended) A composition according to claim 1, wherein the nucleic acid construct further comprises a gene sequence encoding a secretion signal peptide.

9. (Amended) A composition according to claim 1 comprising genes encoding antibody molecules to several different epitopes of the disease-causing agent.

10. (Amended) A composition according to claim 1 comprising a gene-expression library encoding antibodies to the disease-causing agent.

11. (Amended) A composition according to claim 10 wherein the gene expression library encodes single-chain antibody molecules to the disease-causing agent.

12. (Amended) A composition according to claim 1 wherein the encoded recombinant antibody is a virus-neutralising antibody.

13. (Amended) A composition according to claim 12 wherein the encoded virus-neutralising antibody is single chain molecule.

14. (Amended) A composition according to claim 1 including a nucleic acid construct encoding a viral haemorrhagic septicaemia virus VHSV-neutralising monoclonal antibody 3F1H10 with two amino acids substituents in the H-chain gene respectively Asn 35a to Thr and Lys 64 to Thr and with the secretion signal of rainbow trout transforming growth factor (TGF-beta) added to the 5′ end of the gene.

15. (Amended) A composition according to claim 6 wherein when the disease-causing agent is an allergen the antibody molecule is derived from an antibody raised against IgE molecules.

16. (Amended) A composition according to claim 1 wherein the nucleic acid construct is formed from DNA.

17. (Amended) A composition according to claim 1 wherein the composition is in the form of a vaccine, dosage form, cream, ointment, liquid or paint.

18. (Amended) A composition according to claim 17 wherein the composition is for delivery by injection, spray or gene gun.

Please add the following new claims:

-- 21. A composition for protection of an animal against a disease-causing agent, the composition comprising a non-infectious nucleic acid construct encoding a recombinant antibody to that agent wherein the encoded antibody molecule comprises variable domains of immunoglobulin Heavy and Light chain genes linked together by a linker sequence.

22. A composition according to claim 21 wherein the animal is selected from a mammal or a fish.

23. A composition according to claim 21 wherein the animal has a deficient immune system.

24. A composition according to claim 21 wherein the disease-causing agent is selected from a pathogen, an allergen or a toxic substance.

25. A composition according to claim 21 wherein the protection is prophylactic.

26. A composition according to claim 21 wherein the encoded recombinant antibody is derived from an antibody raised against the disease-causing agent.

27. A composition according to claim 21, wherein the nucleic acid construct further comprises a gene sequence encoding a secretion signal peptide.

28. A composition according to claim 21 comprising genes encoding antibody molecules to several different epitopes of the disease-causing agent.

29. A composition according to claim 21 comprising a gene-expression library encoding antibodies to the disease-causing agent.

30. A composition according to claim 29 wherein the gene expression library encodes single-chain antibody molecules to the disease-causing agent.

31. A composition according to claim 21 wherein the encoded recombinant antibody is a virus-neutralising antibody.

32. A composition according to claim 31 wherein the encoded virus-neutralising antibody is single chain molecule.

33. A composition according to claim 21 including a nucleic acid construct encoding a viral haemorrhagic septicaemia virus VHSV-neutralising monoclonal antibody 3F1H10 with two amino acids substituents in the H-chain gene respectively Asn 35a to Thr and Lys 64 to Thr and with the secretion signal of rainbow trout transforming growth factor (TGF-beta) added to the 5′ end of the gene.

34. A composition according to claim 26 wherein when the disease-causing agent is an allergen the antibody molecule is derived from an antibody raised against IgE molecules.

35. A composition according to claim 21 wherein the nucleic acid construct is formed from DNA.

36. A composition according to claim 21 wherein the composition is in the form of a vaccine, dosage form, cream, ointment, liquid or paint.

37. A composition according to claim 36 wherein the composition is for delivery by injection, spray or gene gun.

38. A composition for protection of a fish against a disease-causing agent, the composition comprising a non-infectious DNA construct encoding a viral haemorrhagic septicaemia virus VHSV-neutralising monoclonal antibody 3F1H10 with two amino acids substituents in the H-chain gene respectively Asn 35a to Thr and Lys 64 to Thr and with the secretion signal of rainbow trout transforming growth factor (TGF-beta) added to the 5′ end of the gene.

39. A composition according to claim 38 wherein the protection is prophylactic.

40. A composition according to claim 38 wherein the encoded antibody molecule comprises variable domains of immunoglobulin Heavy and Light chain genes linked together by a linker sequence.

41. A composition according to claim 38, wherein the nucleic acid construct further comprises a gene sequence encoding a secretion signal peptide.

42. A composition according to claim 38 wherein the composition is in the form of a vaccine, dosage form, cream, ointment, liquid or paint.

43. A composition according to claim 38 wherein the composition is for delivery by injection, spray or gene gun.

44. A method of treating an animal comprising administering thereto a composition according to claim 1.

45. A method according to claim 44, wherein said composition mediates expression of a recombinant antibody to the pathogen, allergen or toxin.

46. A method of treating an animal comprising administering thereto a composition according to claim 3.

47. A method of treating an animal comprising administering thereto a composition according to claim 6.

48. A method of treating an animal comprising administering thereto a composition according to claim 21.

49. A method of treating a fish comprising administering thereto a composition according to claim 38.

50. A method of treating an animal with a congenital or acquired imunodefficiency, comprising administration of a number of non-infectious nucleic acid constructs encoding antibodies against a spectrum of disease-causing agents.

51. A method according to claim 44, wherein said animal is a fish or another aquatic animal.

52. A method according to claims 44, wherein said animal is a mammal.

53. A method according to claim 52, wherein said mammal is a human.

54. A method according to claim 50, wherein said animal is a human. --

REMARKS

This Preliminary Amendment is being filed to amend the claims to conform with U.S. practice and to add new claims 21 through 54 drawn to subject matter described throughout the specification and in original claims 19 and 20, now canceled. No new matter has been added by this amendment and entry is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE."**

Respectfully submitted,

/Kathleen A. Tyrrell/

Kathleen A. Tyrrell
Registration No. 38,350

Date: **March 20, 2002**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel claims 19 and 20.

Please amend the claims as follows:

1. (Amended) A ~~pharmaceutical~~ composition for protection of an animal against a disease-causing agent, the composition comprising a non-infectious nucleic acid construct encoding a recombinant antibody to that agent.

2. (Amended) A ~~pharmaceutical~~ composition according to claim 1 wherein the animal is selected from a mammal or a fish.

3. (Amended) A ~~pharmaceutical~~ composition according to ~~either of~~ claim 1 ~~or 2~~ wherein the animal has a deficient immune system.

4. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 wherein the disease-causing agent is selected from a pathogen, an allergen or a toxic substance.

5. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 wherein the protection is prophylactic.

6. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 wherein the encoded recombinant antibody is derived from an antibody raised against the disease-causing agent.

7. (Amended)A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 wherein the encoded antibody molecule comprises variable domains of immunoglobulin Heavy and Light chain genes linked together by a linker sequence.

8. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1, wherein the nucleic acid construct further comprises a gene sequence encoding a secretion signal peptide.

9. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 comprising genes encoding antibody molecules to several different epitopes of the disease-causing agent.

10. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 comprising a gene-expression library encoding antibodies to the disease-causing agent.

11. (Amended) A ~~pharmaceutical~~ composition according to claim 10 wherein the gene expression library encodes single-chain antibody molecules to the disease-causing agent.

12. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 wherein the encoded recombinant antibody is a virus-neutralising antibody.

13. (Amended) A ~~pharmaceutical~~ composition according to claim 12 wherein the encoded virus-neutralising antibody is single chain molecule.

14. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 including a nucleic acid construct encoding a viral haemorrhagic septicaemia virus VHSV-neutralising monoclonal antibody 3F1H10 with two amino acids substituents in the H-chain gene respectively Asn 35a to Thr and Lys 64 to Thr and with the secretion signal of rainbow trout transforming growth factor (TGF-beta) added to the 5' end of the gene.

15. (Amended) A ~~pharmaceutical~~ composition according to ~~any of claims 4 to 11~~ claim 6 wherein when the disease-causing agent is an allergen the antibody molecule is derived from an antibody raised against IgE molecules.

16. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 wherein the nucleic acid construct is formed from DNA.

17. (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 1 wherein the composition is in the form of a vaccine, dosage form, cream, ointment, liquid or paint.

18. A (Amended) A ~~pharmaceutical~~ composition according to ~~any preceding claim~~ claim 17 wherein the composition is for delivery by injection, spray or gene gun.

MONOCLONAL ANTIBODY 3F1H10 NEUTRALISING VHSV (VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS)

The present invention relates to a non-infectious nucleic acid (RNA and DNA) construct constructed to express a recombinant antibody or antibody fragment in a host cell. The antibody molecule confers protection to the host against a pathogen, allergen or toxin. The host may be any animal including a human.

Passive immunization by injection of homologous or heterologous serum-antibodies is routinely used in humans for immunoprophylaxis of people traveling to foreign regions involving risk of exposure to exotic pathogens. In animals a similar strategy may be employed for protection of valuable specimens, but is generally too expensive for routine veterinary use. Passive immunisation of animals against infectious diseases is thus mostly done on an experimental basis with the aim of studying the function of structures such as antibodies *in vivo* and relating the results to *in vitro* experiments.

During the recent decade, diverse technologies for the *in vitro* production of antibodies by the use of recombinant DNA technology has been developed. The smallest functional recombinant antibody combining the actions of the heavy (H) and light (L) polypeptide chains as in the native molecule has proved to be the single chain variable-fragment construct (single chain FV). The single chain FV construct is composed of the variable parts of the H and L chains connected by a flexible spacer region. Such molecules have been used in various studies including virus neutralisation, cancer-immunotherapy and recently also in the form of DNA vaccines where plasmids encoding anti-idiotype single chain FV

antibodies have proved able to induce an antigen-specific immune response. However, direct establishment of protective immunity to infectious diseases by prophylactic treatment with plasmid DNA carrying single chain FV genes encoding protective antibodies has not been described.

An object of the present invention is to provide a non-infectious nucleic acid construct which can produce an antibody molecule *in vivo* thereby conferring immunity to a disease.

A further object of the present invention is to provide a method of establishing immunity against a pathogen.

A yet further object of the present invention is to provide a method of therapy for animals which have a deficient immune system.

An additional object of the present invention is to provide a method of therapy for an animal suffering from an allergic reaction or a method of preventing an allergic reaction.

For avoidance of doubt it should be noted that the word "animal" includes but is not restricted to mammals including humans.

According to an embodiment of the present invention there is provided a nucleic acid construct encoding a recombinant antibody molecule, said construct being adapted for the *in vivo* establishment of a protective immunity to an infectious disease in an animal.

According to a further embodiment of the present invention there is provided a nucleic acid construct encoding a recombinant antibody molecule, said construct is formulated for the *in vivo* prevention of an allergic reaction to an allergen in an animal.

According to a yet further embodiment of the present invention there is provided a nucleic acid construct encoding a recombinant antibody molecule, wherein said construct is formulated for the *in vivo* prevention of a reaction caused by the presence of a toxic substance in an animal.

The term recombinant antibody molecule encompasses a full size antibody, a single chain variable fragment or any part of an antibody which can recognise an antigen. In this connection, conveniently the antibody fragment does not have to be single chain. However, in some embodiments it is single chain.

It has now been found that the intramuscular injection of a nucleic acid construct, in the form of a plasmid, encoding a virus-neutralising single chain antibody fragment can mediate *in vivo* expression of antibodies which protect an animal against a possibly lethal exposure to a virus. This has been established in an experimental model which involves a fish rhabdovirus called viral haemorrhagic septicaemia virus (VHSV) in the rainbow trout (Oncorhynchus mykiss) as a host species.

According to a further embodiment of the present invention there is provided a nucleic acid construct, such as a plasmid, comprising an expression vector and a gene sequence for heavy and/or light chain variable domains of an antibody.

Preferably the heavy and light chain variable domains are linked by a linker sequence in order that they form what is known in the art as a single chain variable-fragment.

It is thought that the antibody fragment as expressed in and secreted from a host cell carrying the vector will act with the same specificity as a natural antibody would in the presence of a substance which it recognises. In this connection, for example, if the heavy and/or light chain variable domain were derived from a monoclonal antibody raised against dengue virus then if dengue virus infected a host who had received a nucleic construct expressing a single chain variable fragment produced from the heavy and light chain of the monoclonal antibody, the fragment would recognise cells infected with the dengue virus or the dengue virus particle itself and bind thereto thereby neutralising or inhibiting the virus and/or giving the host time to mount an immune response against the virus.

In preferred embodiments the expression vector is made for eukaryotic expression and/or is non infectious. For example, a bacterial plasmid, or a smaller DNA fragment carrying the variable fragment antibody gene within a eukaryotic expression operon including regulatory elements such as an enhancer, promoter and polyadenylation signal could be used. Alternatively, stabilised messenger RNA including a positive strand transcript of the variable-fragment antibody gene with translation signals may be employed.

The antibody fragment genes can be cloned by any method known to those skilled in the art, for example from hybridoma cells or directly from B-lymphocytes from immunized individuals. Nucleic acid constructs encoding protective antibody fragments

can be prepared against any important pathogen/disease causing agent in animals including pathogens against which vaccines are not available or have proved insufficient. Furthermore, as a result of veterinary regulations, use of live vaccines may not be allowed. In such cases an alternative prophylactic measure would have to be taken. Such a measure could be the administration of the nucleic acid construct of the present invention. A list of possible pathogens is given below; this list is not intended to be exhaustive.

Viral haemorrhagic septicaemia virus (fish)
Infectious haematopoietic necrosis virus (fish)
Infectious salmon anemia virus (fish)
Infectious pancreatic necrosis virus (fish)
Nodaviruses (fish)
Renibacterium salmoniarum (fish)
Pasteurella (fish)
Ichthyopthtirius mulitifiliis (fish)
NewCastle disease virus (fowl)
Infectious bursal disease virus(fowl)
Bovine respiratory syncytial virus (cattle)
Bovine virus diarrhoea virus (cattle)
Porcine reproductive and respiratory syndrome virus (pigs)
Pseudorabiesvirus (pigs)
Equine herpes virus 1 (horses)
Plasmocytosis virus (mink)
Rabies virus (dogs)
Feline leukemia virus (cats)
Foot and mouth disease (cattle)
Human immune deficiency virus (human)
Hepatitis A virus (human)
Borrelia sp. (human)
Plasmodium sp. (human)

Rabies virus (human)
Epstein-Barr virus (human)

In case of humans with either a congenital or acquired immunodeficiency, vaccines will generally be insufficient. In such cases, administration of a number of nucleic acid constructs according to the present invention encoding antibodies against a broad spectrum of pathogens may be considered.

For the purpose of prevention of allergic relations induced by IgE response, administration of nucleic acid constructs mediating expression of an allergen-specific recombinant antibody may be used to competitively inhibit binding of the allergen to the IgE molecules in the host. Alternatively gene constructs encoding anti-IgE antibodies may be used to interfere with the interaction between IgE and mast cells in the allergic individual.

Administration of antibody gene constructs encoding antibodies to toxins or venoms can be used for the prophylactic treatment of individuals periodically being in high risk of exposure to toxic organisms. The venoms could, for example, be from snakes or spiders.

Conveniently the construct further comprises a gene encoding a signal sequence for the secretion of the product encoded by the gene sequence. The signal sequence will allow the product of the gene sequence to be secreted from a cell in which the gene has been expressed, into the blood so that the product of the gene sequence can circulate therein. For example, the genes for the signal sequence of either rainbow trout transforming growth factor beta (TGF-beta), or murine Ig

kappa-chain can be added to the 5' end of a gene to be administered to the fish. Other secretion signals, preferably of homologous origin to the host species may be employed. Examples of genes which encode proteins which act as secretion signals include the gene for immunoglobulin heavy and light chain secretion signals or other glycoprotein secretion signals. Preferably, the secretion signal should include a proteolytic cleavage site ensuring removal of the signal peptide before secretion of the antibody fragment.

Preferably the construct further comprises a known gene sequence which encodes a short peptide sequence that can be used to identify transfected cells. Such a gene sequence can be attached to the 3' end of the gene. Examples of such a sequence include a human kappa light chain construct or sequence encoding a six histidine residue. In both cases, an antibody specifically recognising the expressed peptide is commercially available.

The construct according to the present invention may be delivered by any suitable method, such as by injection (e.g intramuscularly), by a spray on a mucosa surface (e.g intranasally), by particle bombardment on skin/dermis through use of a gene gun, by electroporation or by uptake by an animal from an aqueous environment. In this connection, the plasmid may be encased in a liposome for administration to an animal. The construct may be administered to the animal topically, through inhalation or orally. For oral administration the construct should be protected from degradation by proper encapsulation.

It is preferred that in a composition or formulation for administration of the constructs there are present genes

encoding the heavy and/or light chain variable fragments against several different epitopes or an variable fragment antibody gene expression library against a given pathogen. In this connection, the various fragments may be provided on one plasmid or they may be provided on several different gene constructs which are all present in the same formulation or other method of administration. In the alternative, each plasmid may have to be administered separately.

The invention also provides for a method for treating an animal, for example a mammal or a fish which comprises administering thereto a plasmid or other nucleic acid construct encoding a protective antibody fragment as previously described.

The invention thus provides for a method of therapy for an animal which has a deficient immune system.

The invention also provides for a therapeutic composition comprising the plasmid as previously described and a pharmaceutically acceptable diluent or carrier therefor. The composition may be formulated such that it is in the form of, for example, a vaccine, dosage form, cream, ointment, liquid or paint.

The invention will now be described by way of illustration only with reference to the following Example and Figures.

Figure 1 shows a schematic drawing of the pCDNA3 plasmid with a single chain antibody (ScAb) gene construct inserted downstream of a strong eukaryotic promoter from cytomegalovirus (CMV). PCDNA3 is a commercially available eukaryotic expression vector (Invitrogen).

Figure 2 shows a culture of EPC cells (passaged fish cells) transfected with a pCDNA3-BU1. BU1 is a ScAb gene construct encoding a recombinant antibody which is able to neutralise the fish pathogenic rhabdovirus, VHSV. BU1 carries a part of the human kappa light chain gene as a residue or tag. Twelve days after the date of transfection the cells were fixed and stained immunochemically using horseradish peroxidase-conjugated rabbit antibody to human kappa light chain (HRP-Rabbit anti-kappa) for the detection of cells containing ScAb. These cells give a positive response and are darker than the remaining cells; and

Figure 3 shows a histological section of muscle tissue sampled from a fish twelve days after intramuscular injection of pCDNA3-BU1. The section was stained immunochemically using HRP-rabbit anti-kappa for the detection of ScAb. Several cells turned out positive (arrow heads) along the regenerating needle track (injection site) arrowed.

Gene Map

The following gene map is the DNA sequence of the construct comprising a single chain antibody gene (BU1) inserted into E.coli pCDNA3 plasmid (Invitrogen) used in the Example described below.

1 cagtgtgcta acatgagggc agtgtgtttg atgctgactg ccttattgat
51 gctggaatat gtgtgccgga gtgaccaggt gcagctgcag gagtcaggac
101 ctggcctcgt gaaaccttct cagtctctgt ctccaccctg ctctgtcact
151 ggctactcca tcaccagtgg ttattactgg acctggatcc ggcagtttcc
201 aggaaataaa ctggaatgga tgggctacat aagctacgac ggtaccaata
251 actacaaccc atctctcaca aatcgaatct ccatcactcg tgacacatct
301 aagaaccagt tttcctgaa gttgaaatct gtgactactg aggacacagc

351 tacatattac tgtgtaagag ggatctacta tggtaacgac tggtttgctt
401 actggggcca agggaccacg gtcaccgtct cctcagaagg caaatcttct
451 ggctctggct ctgaatctaa agtggatgac atcgagctca cccagtctcc
501 tgcctcccag tctgcatctc tgggagaaag tgtcaccatc acatgcctgg
551 caagtcagac cattggtaca tggttagcat ggtatcaaca gaaaccaggg
601 aaatctcctc agctcctgat ttatgctgca accagtttgg cagatggggt
651 cccatcaagg ttcagtggta gtggatctgg cacaaaattt tctttcaaga
701 tcagcagcct acaggctgaa gattttgtaa gttattactg tcaacaactt
751 tacagtactc cgtacacgtt cggaggggag accaagctcg agatcaaacg
801 gactgtggct gcaccatctg tcttcatctt cccgccatct gatgagcagt
851 tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc
901 agagaggcca aagtacagtg gaaggtggat aacgccctcc aatcgggtaa
951 ctcccaggag agtgtcacag agcaggacag caaggacagc acctacagcc
1001 tcagcagcac cctgacgctg agcaaagcag actacgagaa acacaaagtc
1051 tacgcctgcg aagtcaccca tcagggcctg agttcgcccg tcacaaagag
1101 cttcaaccgc ggagagtcat aagttagata tccat

The 301 insert (ScAb gene construct) is encoded by nucleotides 10 to 1125. The coding region nucleotides are 13 to 1122.

The above identified sequence can be found in the Genebank, the Accession Number is AF302092.

Example

Single chain antibody genes were prepared according to the procedure described by McGregor et al; Spontaneous Assembly of Divalent Single Chain Antibody Fragments in E-Coli; Mol. Immunol, February 31(3) pp 219 to 226; 1994. In short, the variable domains of the immunoglobulin H and L chain genes were cloned from hybridoma cell lines producing monoclonal antibodies to the fish pathogenic rhabdovirus viral haemorrhagic septicaemia virus(VHSV). The H and L chain variable domains were linked by a gene sequence encoding a 14

amino acid linker to generate a single chain antibody (ScAb) gene. As a tag to allow specific detection, the human kappa light chain constant domain gene was included at the 3' end of the gene. In order to ensure secretion of the ScAb polypeptides in eukaryotic cells, the nucleotide sequence encoding the 20 amino acid signal peptide of rainbow trout transforming growth factor beta (TGF-beta) was added at the 5' end of the gene.

The gene construct was inserted by blunt-end ligation into the eukaryotic expression vector pCDNA3 (Invitrogen) in the EcoR I site in the polylinker downstream of a cytomegalovirus (CMV) promoter (see Figure 1). As a negative control in transfection experiments with cell cultures and immunoprotection trials in fish, the pCDNA3 plasmid without insert was used. Plasmid DNA was purified from overnight cultures of *E.coli* by use of commercial kits for anion-exchange chromatography as recommended by the supplier (Qiagen).

Other molecular biology procedures used were as followed by Sambrook et al in Molecular Cloning; A Laboratory Manual, Second Addition, Cold Spring Harbor Laboratory, USA, (1989). The variable domain genes from a hybridoma cell line secreting the VHSV-neutralising monoclonal antibody 3F1H10 were used. Cloning and sequencing of the variable domain genes has already been described. In the case of antibody 3F1H10, two amino acids substitutions were made to the H-chain (Asn35a to Thr and Lys64 to Thr). The ScAb carrying the variable domains of antibody 3F1H10 was called BU1.

Passaged fish cells designated (EPC) were transfected with an anionic transfection reagent (Superfect, Qiagen,. Four to six

days after transfection cell culture supernatant were harvested and analysed for antibody reactivity to VHSV. After removal of the supernatant, the cells remaining attached to the bottom of the cell culture wells were fixed in 80% cold acetone and stained by immuno-peroxidase using horseradish peroxidase-conjugated rabbit antibody to human kappa light chain (HRP-Rabbit anti-kappa)(DAKO, Denmark) in order to detect cells expressing ScAb. The effect of transfection on the susceptibility of the cell cultures to VHSV different doses of live VHSV was examined by adding the different doses to wells with cultures of transfected cells four days after transfection and the development of cytopathogenic effects (CPE) was recorded thereafter.

Injection of Plasmid DNA into Fish

Disease free rainbow trout fingerlings, average weight 4.5g, were anaesthetised with 0.001% benzokaine and given two 25μl injections of 20 μg plasmid DNA each, in the epaxial muscles below the dorsal fin. The fish were afterwards kept in groups of approximately 150 individuals in 120-liter tanks supplied with running tap water. The fish were fed *ad libitum* with commercial fish feed. Mean water temperature was 16°C. Injected plasmid constructs included the pCDNA3 vector without insert, and pCDNA3 carrying the ScAb BU1 gene construct (pCDNA-BU1) respectively.

Immunohistochemical Analysis for Expression of ScAb in Injected Fish

Twelve days after injection of plasmid DNA, 10 fish were sampled for each plasmid construct. After termination of the fish a section of muscle tissue was excised from the site of injection. The tissue was fixed in 10% phosphate buffered formalin and analysed by immunohistochemistry. Horseradish

peroxidase-conjugated rabbit immunoglobulin (Ig) to human kappa light chain (HRP-rabbit anti kappa) (Dako, Denmark) was used for detection of expressed ScAb.

Sampling of Plasma from Fish

Blood samples were collected 12 days after injection of plasmid DNA from fish not exposed to VHSV. Due to the small size of the fish, sampling was performed with heparin-treated capillary tubes after cutting off the posterior fin of fully anaesthetised fish. The fish were terminated immediately afterwards. The blood samples were centrifuged at 5000 xg and plasma samples were collected and stored at -80°C until analysed.

Serological Examination for VHSV-reactive ScAbs

Supernatant from transfected cell cultures and plasma samples from DNA-injected fish, were examined for anti-VHSV reactive ScAbs by a plaque-neutralisation (50% PNT) assay and by an enzyme-linked immunosorbent assay (ELISA).

The ELISA assay was performed in 96-well microtitre plates coated with purified VHSV. Bound ScAb's were detected with HRP-Rabbit anti-kappa. In order to demonstrate that the virus-neutralising activity detected in the trout plasma was due to the ScAbs produced by the fish and not by trout antibodies, two variants of the 50% PNT assay were also applied. One variant included parallel examination of the neutralising activity against the virulent VHSV3592B and a neutralisation resistant variant of VHS 3592B (VHSV DK-3542B) selected by cultivating virus in the presence of the neutralising Mab 3F1A2 which is highly similar to Mab 3F1H1C. The other variant involved pre-incubation of the trout plasma with rabbit antibodies to human kappa light chain or with

rabbit antibodies to trout immunoglobulin before incubation with virus. The 50% PNT microplate assay was performed as described by Olesen and Jørgensen in Detection of neutralising antibody to Egtved virus in rainbow trout by plaque neutralising with complement addition, J. Appl Ichthyol, Volume 2, pages 35 to 41.

Immunoprotection Trials in Fish

Eleven days after injection of the plasmid, groups of fish were exposed to (challenged with) the virulent VHSV DK-3592B isolate by immersion in water containing 100 000 50% tissue-culture infective doses per ml. Challenge was performed in 8-liter aquaria with 25-31 fish in each. Three replicate aquaria was included for each plasmid construct. Dead fish were afterwards daily recorded and collected. Dead fish from all tanks were analysed virologically for the presence of VHSV. Mean water temperature was 16°C from the time of injection to immediately before challenge. At challenge, the fish were adapted to a water temperature of 12°C and this temperature was kept throughout the 20 day challenge period.

Immunochemical Detection of Expressed ScAb in cell Culture and in Fish

It was found that after immuno-peroxidase staining using the HRP-rabbit anti-human kappa, single cells expressing ScAb could be detected in EPC cell cultures transfected with the plasmid construct pCDNA3-BU1 (Fig. 2), whereas no positive cells were found in cultures transfected with pCDNA3 without insert. Similarly, expression of ScAb could be demonstrated in muscle sections from injected fish (Fig. 3). No positive cells were found in fish injected with pCDNA3 without insert.

Interference of ScAbs with propagation of VHSV in Cell Culture

When monolayers of epithelial cell line of carp cell cultures were inoculated with VHSV four days after transfection, development of cytopathogenic effect (CPE) as a result of multiplication of VHSV was highly different in cultures transfected with pCDNA3 compared to cell cultures transfected with pCDNA3-BU1. In the latter case only certain plaques of cells became infected and died and there was no further development of CPE in the 8-day observation period. In contrast, when cultures transfected with pCDNA3 were inoculated, all cells became infected and were destroyed within 3-6 days as in a normal propagation of VHSV in EPC cells (Table 1).

Table 1. Susceptibility of transfected EPC cell cultures to VHSV

Plasmid Construct used for Transfection	**Cytopathogenic effect upon inoculation with VHSV***
pCDNA3	Complete destruction of cell layer
pCDNA3-BU1	Plaques

* Concentrations of VHSV: 10^2-10^3 TCID-50/ml cell culture medium.

Detection of ScAbs to VHSV in the Fish

When the plasma from injected fish was analysed by ELISA for ScAbs recognising VHSV, a strong reaction was found in plasma from fish injected with pCDNA3-BU1. No reactivity was detected in plasma from fish injected with pCDNA3 without insert. As indicated in Table 2, the limited amounts of

plasma available made it necessary to perform the analysis on pools of five individuals. The 50% PNT analysis was performed on individual plasma samples. All 10 individuals injected with pCDNA3-BU1 neutralised VHSV, whereas no neutralising activity was detected in plasma from fish injected with the pCDNA3 (Table 3). When plasma from fish injected with pCDNA3-BU1 was preincubated with Rabbit anti-human kappa before testing in 50% PNT, the neutralising activity was eliminated, whereas no effect was observed upon pre-incubation with normal rabbit serum or with rabbit serum to trout Ig (Table 4). The neutralising activity of a positive trout serum control was unaffected by pre-incubation with normal rabbit serum and with rabbit anti-human kappa, but was highly reduced upon pre-incubation with rabbit serum to trout Ig (Table 4). As with the parent monoclonal antibody 3F1H10, plasma samples from fish injected with pCDNA3-BU1 could neutralise the virulent VHSV DK-3592B isolate, but not a neutralisation escape-mutant (not shown).

Table 2. Antibody reactivity in fish plasma: ELISA

Fish No. *	Injected Plasmid	Reactivity with VHSF (A-496 nm)	
		Dilution: 1/10	Dilution: 1/80
36529	pCDNA3	0	0
36686		0	0
36844	pCDNA3-BU1	3	1
16-20		3	1

* The plasma samples were analysed in pools of 5 individuals.

Table 3. Antibody reactivity in fish plasma: Neutralisation of VHSV

Fish No. *	Injected Plasmid	PNT-titres **
36534	pCDNA3	<10
36849	pCDNA3-BU1	160-640

* Plasma samples were analysed individually.

** Titres represent the reciprocal value of plasma dilutions reducing the number of plaques to approximately 50% compared to a control well without antibody/plasma.

Table 4. Effect of preincubation of trout plasma with rabbit antibodies on PNT-titres*

Fish No.	Injected Reagent	PNT-titres		
		Normal rabbit	Rabbit to human chain kappa	Rabbit to trout Ig
21-30 (1 pool)	pCDNA3-BU1	640	<40	320-640
Positive trout serum A7.1	Killed VHSV	>10240	>10240	320

* In order to allow detection of neutralising trout antibodies, trout complement was included as described above.

Infection Trial

When challenged with VHSV DK-3592B 11 days after injection of plasmid DNA, most of the fish injected with pCDNA3-BU1 survived whereas high mortalities were observed among fish injected with pCDNA3 (Table 5).

Table 5. Protection against VHSV

Injected Plasmid	Accumulated mortality 20 days post challenge (mean of triplicate tanks)
pCDNA3	81%
pCDNA3-BU1	6%

To our knowledge, this is the first report demonstrating establishment of protective immunity to an infectious pathogen in higher vertebrates by administration of genes encoding pathogen specific single chain FV antibodies. The protective activity of the pCDNA-BU1 construct fully correlated with the occurrence of neutralising anti-VHSV ScAbs in the plasma of injected fish, and although involvement of non-specific mechanisms cannot be completely excluded, it appears likely that the produced BU1 ScAb has been the major cause of protection following injection of the pCDNA3-BU1 plasmid DNA. Accordingly, in a later experiment including challenge of the fish with a virus isolate not recognised by the recombinant antibody fragment encoded by pCDNA-BU1, no protection was obtained.

In contrast to DNA-vaccines, including anti-idiotype vaccines, the administration of plasmid borne genes in this instance do

not involve specific activation of the immune system in the individual. The principle is simply that single chain FV antibody polypeptides produced by the cells that take up the administered plasmid will be systemically distributed by the body fluids and protect the individual if infection with the pathogen occurs. This corresponds to the mechanism of prophylaxis against infectious diseases in humans through administration of antiserum or immunoglobulin from immunised donors or animals, but without side effects such as risk of concomitant transfer of infectious diseases or induction of hypersensitivity following repeated administrations. In order to avoid the pathogen variability overcoming the immunity established by the plasmid, practical use may involve administration of plasmids encoding genes of single chain variable fragments to several different epitopes of the pathogen or single chain FV antibody gene-expression library towards a given pathogen.

The principle of genetic immunoprophylaxis according to the invention can be extended to mammals and to humans in particular as it is a valuable tool for transient protection of individuals such as travelers against exposure to pathogens or toxins where no efficient vaccines are available. Similarly, the invention may be used for induction of the synthesis of antibodies of a desired specificity for use in immunodeficient individuals. Also the nucleic acid construct of the present invention could be used in individuals that produce an allergic response to certain allergens, such as pollen. In this connection, production or induction of antibody fragments to those allergens may be used for prevention of an allergic reaction.

Beside the prophylactic aspects of the invention, plasmid constructs carrying genes encoding pathogen/disease antigen specific single chain FV antibodies are of therapeutic use in certain diseases wherein the host immune system itself is unable to produce antibodies with the necessary activity.

CLAIMS:-

1. A pharmaceutical composition for protection of an animal against a disease-causing agent, the composition comprising a non-infectious nucleic acid construct encoding a recombinant antibody to that agent.

2. A pharmaceutical composition according to claim 1 wherein the animal is a mammal or a fish.

3. A pharmaceutical composition according to either of claims 1 or 2 wherein the animal has a deficient immune system.

4. A pharmaceutical composition according to any preceding claim wherein the disease-causing agent is a pathogen, an allergen or a toxic substance.

5. A pharmaceutical composition according to any preceding claim wherein the protection is prophylactic.

6. A pharmaceutical composition according to any preceding claim wherein the encoded recombinant antibody is derived from an antibody raised against the disease-causing agent.

7. A pharmaceutical composition according to any preceding claim wherein the encoded antibody molecule comprises variable domains of immunoglobulin Heavy and Light chain genes linked together by a linker sequence.

8. A pharmaceutical composition according to any preceding claim, wherein the nucleic acid construct further comprises a gene sequence encoding a secretion signal peptide.

9. A pharmaceutical composition according to any preceding claim comprising genes encoding antibody molecules to several different epitopes of the disease-causing agent.

10. A pharmaceutical composition according to any preceding claim comprising a gene-expression library encoding antibodies to the disease-causing agent.

11. A pharmaceutical composition according to claim 10 wherein the gene expression library encodes single-chain antibody molecules to the disease-causing agent.

12. A pharmaceutical composition according to any preceding claim wherein the encoded recombinant antibody is a virus-neutralising antibody.

13. A pharmaceutical composition according to claim 12 wherein the encoded virus-neutralising antibody is single chain molecule.

14. A pharmaceutical composition according to any preceding claim including a nucleic acid construct encoding a viral haemorrhagic septicaemia virus VHSV-neutralising monoclonal antibody 3F1H10 with two amino acids substituents in the H-chain gene respectively Asn 35a to Thr and Lys 64 to Thr and with the secretion signal of rainbow trout transforming growth factor (TGF-beta) added to the 5' end of the gene.

15. A pharmaceutical composition according to any of claims 4 to 11 wherein when the disease-causing agent is an allergen the antibody molecule is derived from an antibody raised against IgE molecules.

16. A pharmaceutical composition according to any preceding claim wherein the nucleic acid construct is formed from DNA.

17. A pharmaceutical composition according to any preceding claim wherein the composition is in the form of a vaccine, dosage form, cream, ointment, liquid or paint.

18. A pharmaceutical composition according to any preceding claim wherein the composition is for delivery by injection, spray or gene gun.

19. A method of treating an animal comprising administering thereto a pharmaceutical composition as claimed in any of claims 1 to 18.

20. A pharmaceutical composition according to any preceding claim, for use to confer protection against a disease caused by a pathogen, an allergen or a toxin.

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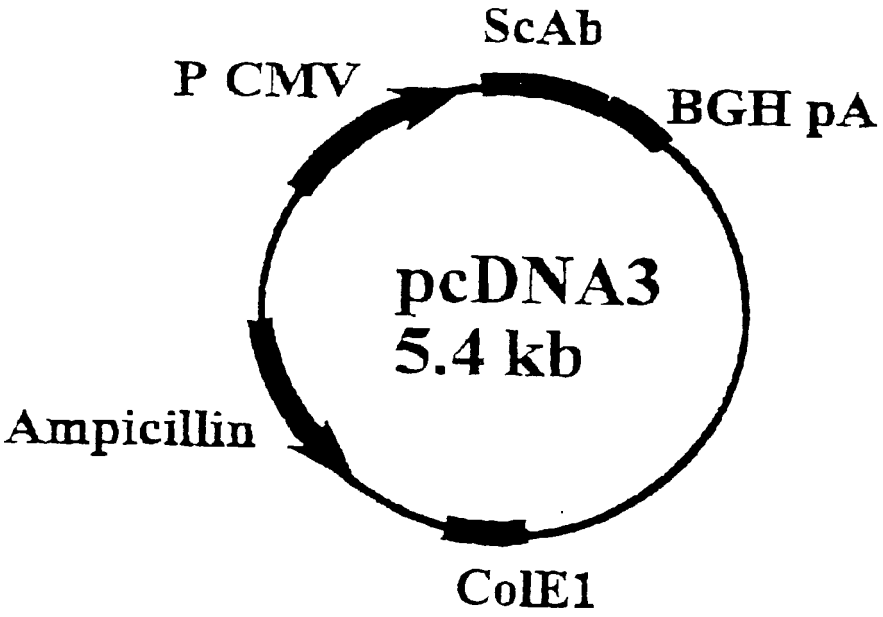
(54) Title: MONOCLONAL ANTIBODY 3F1H10 NEUTRALISING VHSV (VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS)

(57) Abstract: The present invention relates to a non-infectious nucleic acid (RNA and DNA) construct constructed to express a recombinant antibody or antibody fragment in a host cell. The antibody molecule confers protection to the host against a pathogen, allergen or toxin. The host may be any animal including a human.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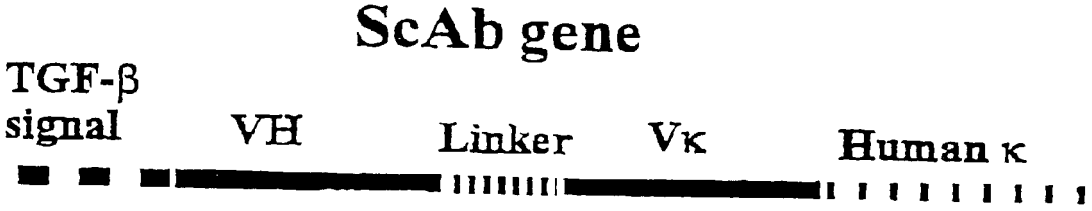
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Human κ

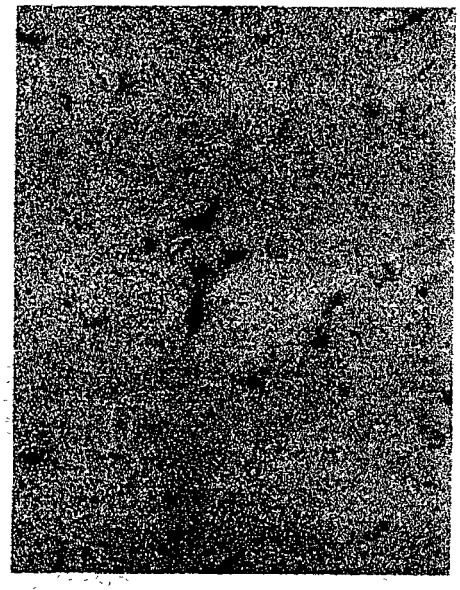

Figure 2

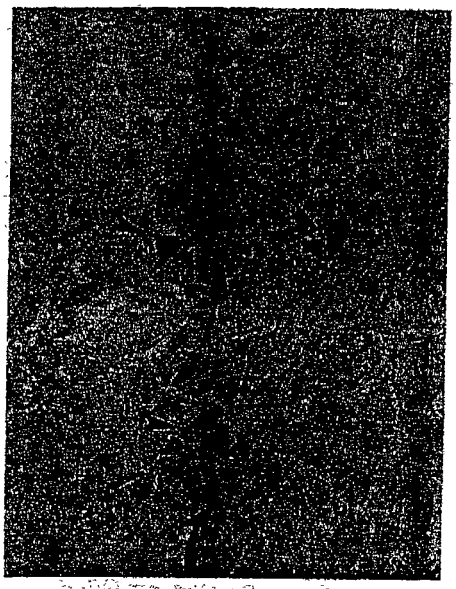

Figure 3

Docket No.
ABLE-0021

#3

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODY 3F1H10 NEUTRALISING VHSV (VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS)

the specification of which

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☐ is attached hereto.

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and was amended on _____
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I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

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Prior Foreign Application(s)			Priority Not Claimed
PA 1999 01329	**DK**	**20 September 1999**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
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(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
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